27. A beverage, food or feed composition comprising the compound according to claim 18 and a liquid, food or feed acceptable carrier.

28. The compound according to claim 18, which is used as a medicament.

29. The compound according to claim 18, which comprises one of the following amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4 and SEQ ID NO. 5.

REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

Initially, Applicants and their undersigned representative wish to express their sincere appreciation to the Examiner for his courtesy during the telephone interviews.

With respect to the objections to the drawings, Applicants respectfully request that the objections be held in abeyance until after an indication of allowable subject matter.

With respect to Applicants' claim for foreign priority, Applicants have amended the specification to indicate the status of the priority documents as per the Examiner's request. Applicants also note that they will be submitting a certified copy of the priority document in the future.

The specification has been carefully reviewed and editorial changes have been effected. All of the changes are minor in nature and therefore do not require extensive discussion. Specifically, the specification headings have been amended in conformance with U.S. practice. Further, other corrections to the specification requested by the Examiner has also been effected.

Claims 1, 3-7, 9-12 and 17 have been cancelled without prejudice and replaced with new claims 18-29. Further, claim 15 has been amended to depend on new claim 18. Support for the claim amendment and new claims is readily apparent from the teachings of the specification and the original claims.

Applicants wish to note that unless specifically recited in the arguments below, the changes to the claims are merely editorial in nature and should not be construed to narrow the scope of the claims. It should be noted that some changes have also been effected to broaden the scope of the claims. Applicants believe that the effect of the changes to the claims should be evident on its face and thus, require no further comment.

With regard to the restriction requirement, Applicants hereby affirm the election of rejoined Groups I and II. It is noted that this election is made by the Applicants while retaining their right to file a divisional application directed to the non-elected subject matter with the protection afforded by 35 USC 121.

Claims 15 and 16, directed to non-elected subject matter, remain in the application. It is requested that they be permitted to remain in a dormant condition pending the filing of a divisional application. Applicants note that claim 15 has been amended to depend on base claim 18 for possible rejoinder under *In re Ochiai*. It is noted that Applicants might request that the non-

elected claims in Groups III and IV (claims 15 and 16) be rejoined upon allowance of the product claims in Groups I and II (claims 1, 3-7, 9-12 and 17, rewritten as new claims 18-29).

To update the list of attorneys authorized to prosecute this case before the Patent and Trademark Office, a Associate Power of Attorney is enclosed herewith.

With regard to the rejections of claims 1, 3-7, 9-12 and 17 under 35 U.S.C. § 112, first paragraph, as set forth in items 9 and 11 of the Official Action, these rejections are deemed to be untenable and are thus respectfully traversed.

The Examiner has rejected the claims under 35 U.S.C. § 112, first paragraph, since the Examiner believes that the specification does not enable and does not provide a written description of a compound containing B cell epitope of the *major antigen of Aspergillus fumigatus, the staphylococcal B enterotoxin (SEB), the bovine β-lactoglobulin and T cell epitope of diphtheria, mycobacterium, or measles virus antigens* wherein the compound is used as a *pharmaceutical composition, cosmetical composition, beverage, food and/or feed composition for prevention or treating allergy*. Further, the Examiner also specifically notes that that the specification does not enable and does not provide a written description of a compound consisting of *an antibody secreted by a B cell of a non-atopic individual* and *at least one antigenic determinant of an antigen different from said allergen which triggers T cell activation*. However, from the Examiner's comments and reasoning, Applicants respectfully submit that the Examiner is mistaken in this regard.

The teachings of the specification clearly enable one skilled in the art to practice the invention without undue experimentation. Further, the claims when read in light of the teachings

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of the specification and the knowledge in the art, clearly convey to one skilled in the art that the inventors, at the time of the application was filed, had possession of the claimed invention.

To assist the Examiner in attaining a better understanding of the present invention, Applicants wish to review and explain the key concepts of the present invention.

The claimed compound consists of two parts, a universal T cell epitope and a allergen-derived B cell epitope.

1. T cell epitope

The T cell epitope used in Examples 1-3 of the specification is an universal epitope which is known to be recognized by human beings after vaccination with tetanus toxoid (*see Panina-Bordignon et al., "Universally immunogenic T cell epitopes: promiscuous binding to human MHC class II and promiscuous recognition by T cells", Eur J. Immunol.1989, 19:2237,* submitted herewith and cited in PTO-1449). To confirm this, peripheral blood cells from two patients allergic to Der p 2 and from two non-allergic controls were prepared. B cells were transformed by Epstein-Barr virus infection and activated by incubation with a fibroblast cell line expressing human CD40L. These B cell lines were used to present the tetanus toxoid peptide 830-844 to autologous T cells. To this end, irradiated B cells and T cells were incubated for 6 days in the presence of the tetanus toxoid peptide. Radio-labeled thymidine was then added to the cultures for 18 hours and the radioactivity associated with T cells counted. It can be seen from Figure 1 enclosed herewith that T cells from the two Der p 2 allergic individuals (Al and A2) as well as from the two normal controls (NA1 and NA2) proliferate well above the level of proliferation observed for the control wells in the absence of the peptide.

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It can therefore be clearly concluded that the T cell moiety included in the claimed compound used to treat allergic diseases *is well recognized by allergic and non-allergic subjects*.

2. B cell epitope: Differences between allergic and non-allergic individuals.

Allergic and non-allergic individuals produce antibodies that recognize distinct regions of allergens. The experiments illustrated in Figures 3 and 4 of the patent application, which made use of peptides covering the 7 to 39 region of Der p 2, have now been extended to cover the entire Der p 2 sequence. Thus, plates were coated with synthetic, 15 amino acid-long peptides (peptides 1 to 12) covering the entire sequence of Der p 2 (1 to 129), each peptide overlapping by 5 residues with the two adjacent ones. Total IgG fractions prepared from the serum of three Der p 2 sensitive (atopics) and three non-allergic subjects (non-atopics) were incubated with the plates and the binding of specific IgG was detected by addition of a human Fc-gamma-specific reagent.

It can be seen from Figure 2 enclosed herewith that non-atopic individuals recognize B cell epitopes scattered over the entire Der p 2 molecule, while Der p 2-sensitive individuals recognize only peptides located towards the amino and carboxy-terminal ends of Der p 2. The latter are part of a conformational epitope brought together by a disulphide bridge linking the two ends of the molecule.

It can therefore be clearly concluded that *antibodies made by non-atopic individuals recognize preferentially sequential epitopes scattered over the entire molecule as compared to allergen-sensitive individuals producing antibodies towards conformational and discontinuous epitopes*. It therefore follows that *virtually any sequential epitope on an allergen that is no part of a conformational epitope can serve for the production of a compound suitable for*

administration, provided that the epitope is located in a random coil region of the protein and/or is readily accessible for antibody binding at the surface of the allergen molecule.

3. B cell epitope: Accessibility of the epitope included in the claimed compound.

The 3-D structure of the allergen Der p 2 has been elucidated (*see Mueller GA et al., "Tertiary structure of the major house dust mite allergen Der p 2: sequential and structural homologies", Biochemistry 1998, 37: 12707-12714*, submitted herewith and cited in PTO-1449) . It is now readily apparent that residues 15 to 35 are part of a random coil region which protrudes at the surface of the molecule and is therefore readily accessible to antibodies. Besides, being a region without a fixed secondary structure such as an alpha helix or a beta sheet, this region constitutes a prominent binding site for antibodies (*see Goodman JW, "Immunogens & antigens In: Stites dp, Terr Al, Prslow TG", editors Basic & Clinical Immunology, Eighth ed. Stamford: Appleton & Lange, 1994, p. 53-55*, submitted herewith and cited in PTO-1449).

Mice belonging to different genetic backgrounds produce antibodies towards peptide 11 to 35 (*see Wu B. et al., "Major T cell epitope-containing peptides can elicit strong antibody responses", Eur J. Immunol 2000, 30: 291*, submitted herewith and cited in PTO-1449). The experiments shown in Figure 3 of the patent application and in Figure 2 enclosed herewith clearly show that non-allergic individuals recognize the same Der p 2 region.

The physico-chemical characteristics of the 15-35 region of Der p 2 and the fact that this region is recognized by all mouse strains and non-allergic individuals makes it highly likely that *atopic patients injected with a claimed compound containing at least part of this region will*

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produce antibodies specific to it, which will then cross-react with the native full-length Der p 2 allergen.

4. B cell epitope: Absence of binding of anti-Der p 2 IgE antibodies to the claimed compound.

The claimed compound used for therapy should not be recognized by IgE, antibodies made by allergic individuals, so as to avoid any risk of anaphylactic reaction. Figure 2 of the patent application already shows that IgE of Der p 2 allergic individuals do not bind to synthetic 12-mer peptides covering sequence 7 to 39.

To further confirm that sequence 11-35, or more particularly sequence 21-35 is not recognized by IgE antibodies, a recombinant polypeptide was prepared containing a quadruplicate of sequence 21-35. This polypeptide, called B4, was used in direct binding assays to determine whether it could be recognized by IgE antibodies of allergic individuals. Thus, polystyrene plates were coated by incubation with B4. Dilutions of serum containing anti-Der p 2 IgE antibodies were applied to the plates and the binding of IgE antibodies was detected by addition of either a human IgG- or IgE-specific reagent. It can be seen from Figure 3 enclosed herewith that no IgG or IgE binding is observed when the serum from four unrelated Der p 2-sensitive patients was applied at a ½ dilution. A monoclonal antibody (76B) specific for p21-35 shows, however, a strong binding, indicating that the B4 protein was present and well accessible on the microtiter plate.

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It can therefore be clearly concluded that *region 21-35 is not recognized by IgE antibodies even when 21-35 is presented in the form of a polypeptide, which allows conformation-dependent epitopes to be created.*

5. B cell epitope: Competition between anti-Der p 2 IgE antibodies and peptide 21-35 IgG

The claimed compound for use as an immunotherapeutic agent directs antibody production towards a discrete region of the molecule to the detriment of antibodies generated towards other epitopes (see Figure 1 of the patent application). The data provided below (see Figure 5 enclosed herewith) indicate that such focalization results in a very significant reduction in the production of IgE antibodies towards the native full-length allergen. Apart from its effect on the production of IgE antibodies, the focalization of the immune response could also elicit antibodies capable of competing with IgE binding to distant determinants of the allergen.

An experiment was therefore set up to determine whether IgG antibodies generated in a mouse model towards the claimed compound could interfere with the binding of human anti-Der p 2 IgE antibodies. Thus, mice were immunized with peptide 21-35 in order to produce specific antibodies. The serum of such mice is shown to bind to peptide 21-35 and to Der p 2 in direct binding assays. Different dilutions of mouse serum are mixed with the serum of Der p 2 allergic individuals and the mixtures added to Der p 2 coated plates. After washing, the binding of human IgE antibodies to Der p 2 is assessed by addition of a human IgE-specific reagent. It can be seen from Figure 4 enclosed herewith that a dose-dependent inhibition of IgE binding is observed upon

mixing with mouse serum. The horizontal axis shows the inverse of the serum dilution starting from 1/128.

It can therefore be clearly concluded that *IgG antibodies elicited towards the compound can inhibit the binding of IgE antibodies directed towards distant epitopes of Der p 2*. Since it is anticipated that patients will produce antibodies towards the claimed compound for the reasons noted above, *such antibodies would also be able to inhibit the binding of human IgE antibodies to epitopes located at a distance of region 21-35*.

Furthermore, it is important to emphasize that although most of the experimental work described in the present patent application has been carried out by using Der p 1 and Der p 2, which are two main allergens of the house dust mite, *D. Pteronyssinus*, **data reported by other research groups in this art provide evidence showing that allergic and non-allergic individuals produce antibodies against distinct epitopes of allergens**.

For example, the binding of a given allergen to pools of IgG-antibodies prepared from allergic individuals is only partly inhibited by the addition of IgG-antibodies from non-allergic individuals and vice versa. This experimental data indicate that the precise epitopes recognized by antibodies made by allergic versus non-allergic individuals are different.

In addition, digestion of allergen by proteolytic enzymes significantly reduces the binding of antibodies from allergic individuals but not that of non-allergic donors.

Data are now available that show the same phenomena with allergies as diverse as Der p 1, β-lactoglobulin (*see Duchateau J. et al., "Anti-betalactoglobulin IgG antibodies bind to a*

specific profile of epitopes when patients are allergic to cow's milk proteins", Clin. Exp. Allergy, 1998, 28: 824, submitted herewith and cited in PTO-1449) and phospholitase A2.

Thus, it can be clearly concluded that peptides, which fulfil the criteria of claim 1 in the present patent application, can be easily identified without undue experimentation and that therapeutically active compounds based on such peptides for allergies different from Der pII can be prepared. Thus, Applicants strongly believe that the claimed compounds can be obtained without undue experimentation based on the teachings of the specification and the general knowledge of a person skilled in the art.

Furthermore, Applicants wish to emphasize and further review the biological effects of the administration of the claimed compound which were obtained and evaluated by the inventors.

As stated in the comments above, two types of biological responses was delineated:

1. The focalisation of the antibody response towards the region of the allergen encompassed by the claimed compounds, and
2. The influence of the compound administration in antibody response to distance epitopes of the allergen, and in particular, the effect of IgE antibody production.

A. <u>Focalization</u>

The focalization of the immune response towards peptide 21-35 is described in Figure 1 of the present patent application. Mice immunized with peptide 21-35 after being primed with Der p 2 deviate their antibody response towards the region containing amino acids 21 to 35, and this effect resists further administration of the native Der p 2.

An additional experiment was carried out to determine more precisely the proportion of antibodies directed towards peptide 21-35 and to other Der p 2 epitopes. Thus, mice were immunized with native Der p 2 by SC injections. The animals were then distributed among three

groups for immunization with either a plasmid vector (pCIneo) containing an insert coding for a polypeptide made of a T cell epitope of tetanus toxoid (similar to that used in Example 1 of the present specification) and a duplicate of peptide 21-35 containing an isoleucine to alanine substitution as in Example 5 of the present patent application (group 1). Mice of group 2 were injected with the same vector without the insert and mice of group 3 receive buffer instead of the vector.

At the end of the immunization period, mice were sacrificed and the spleen prepared for fusion with a myeloma cell line. The frequency of precursors corresponding to the number of supernatants containing antibodies to peptide 21-35 or to other epitopes of Der p 2 was then calculated. Mice of groups 2 and 3, receiving either the vector alone or buffer, produced 71% and 67% of antibodies to peptide 21-35, while mice belonging to group 1 receiving the vector with the full insert had 89% of their antibodies directed towards peptide 21-35. This experiment therefore shows that *not only does the peptide 21-35 region of Der p 2 contain a major B cell epitope, but also that the vast majority of anti-Der p 2 antibodies are now directed towards peptide 21-35*.

B. Effect of compound administration on antibodies to other Der p 2 epitopes

In order to evaluate the effect of compound administration to the production of antibodies directed towards determinants located at distance on the Der p 2 molecule, two groups of mice were injected IM with a plasmid vector (pCineo) containing a full insert of the compound (group 1) or with the plasmid vector alone (group 2). All mice were then injected with native Der p 2.

For this experiment, the level of IgG1 antibodies to Der p 2 was assessed, as IgG1 is equivalent to IgE in the mouse model, namely the two antibody isotypes are produced by activation of interleukin 4 - producing T helper cells. Thus, as shown in Figure 5 enclosed herewith, the level of anti-Der p 2 IgG1 was shown to be 75% lower in the group injected with the plasmid vector containing the full insert (lozenge symbols).

It can therefore be clearly concluded that injection of the claimed compound significantly reduces the production of IgE antibodies to determinants of Der p 2 located at distance of the peptide 21-35.

Thus, from this additional information, it can also be clearly concluded that the compounds of the present invention will illicit an immune response in human beings, as well as in a mouse model. This must be the case because the T cell epitope is universal and is shown to be recognized by T cells of atopics as well as non-atopic individuals.

Any B cell epitope of an allergen may be suitable for the production of a compound used for the treatment of allergy, provided that it is sequential and located in a random coil region accessible for antibody binding (the peptide 21-35 fulfills these characteristics).

In addition, the compounds described in the present patent application will be safe for human administration, because the B cell moiety is not recognized by human IgE antibodies, even when used as a polypeptide containing four repetitive units.

Besides, the serum IgG antibodies of mice immunized towards the peptide 21-35 strongly inhibit the binding of human IgE antibodies to all IgE-binding epitopes of the full length allergen

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Der p 2, indicating that an immune response towards a distant region of an allergen can prevent the binding of IgE to other parts of the molecule.

Thus, in light of the above, the application of allergens other than Der p 2 such as the major antigen of *Aspergillus fumigatus*, staphylococcal B enterotoxin (SEB) and the bovine β-lactoglobulin is clearly support by the teachings of the specification and the general knowledge in the art that all allergents tested so far exhibit distinct epitopes recognised by allergic versus nonallergic individuals.

Therefore, in view of the above arguments and explanations, Applicants believe that the rejections under 35 U.S.C. § 112, first paragraph, can no longer be sustained and should be withdrawn.

With regard to the rejection of claims 1, 3-7, 9-12 and 17 under 35 U.S.C. § 112, second paragraph, this rejection is deemed to be untenable in view of the wording of the new claims and is thus respectfully traversed. Specifically, the terms "compound" and "and/or" have been amended to "isolated compound" and "or", respectively. Further, the articles "A" or "The" have been added in the new claims. Thus, in view of the new claims, this rejection can not be sustained and should be withdrawn.

With regard to the rejection of claims 1, 3-7, 9 and 12 under 35 U.S.C. § 102(b), this rejection is deemed to be untenable and is thus respectfully traversed.

To constitute anticipation of the claimed invention, a single prior art reference must disclose each and every material element of the claim. Here, in this case, Bixler et al. do not teach or suggest a compound consisting of (a) at least one allergen antigenic determinant which is

recognised by a B cell or an antibody secreted by a B cell of ***a non-atopic individual*** to said allergen and (b) at least one antigenic determinant of an antigen different from said allergen which triggers T cell activation.

Thus, since Bixler et al. do not teach or suggest a material limitation ("**a non-atopic individual**") of the claims, this rejection can not be sustained and should be withdrawn.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

In view of the foregoing amendments and remarks, it is respectfully submitted that the Application is now in condition for allowance. Such action is thus respectfully solicited.

If, however, the Examiner has any suggestions for expediting allowance of the application or believes that direct communication with Applicants' attorney will advance the prosecution of this case, the Examiner is invited to contact the undersigned at the telephone number below.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claim 15 has been amended as follows:

15. (Amended) A method for preventing or treating an allergy or a disease of allergic origin, which comprises administering the compound according to claim [1] 18, to a patient in need thereof.

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Bxl - 7 May 1999

P.UCB.09/EP (EXT)

ALLERGEN VACCINE

Version with Markings to Show Changes Made

COMPOUND AND METHOD FOR THE PREVENTION AND/OR THE TREATMENT OF ALLERGY

This application claims Foreign Priority under 35 USC§119 (a-d) of EPO Application No 98870178 filed July 30, 1998

1. Field of the invention

The present invention is related to a new compound and a new method for the prevention and/or the treatment of allergy and/or diseases of allergic origin, particularly immediate hypersensitivity allergy.

Background of the invention 2. Description of the Related Art

Immediate hypersensitivity is a form of allergic reaction which develops very quickly, namely within seconds or minutes of exposure of the patient to the causative allergen. This immediate reaction can be followed by a second reaction of delayed onset that can lead to inflammatory changes in the target organ and manifests itself by chronic symptoms such as asthma or atopic dermatitis.

Immediate hypersensitivity is mediated by antibodies belonging mainly, but not exclusively, to the IgE isotype. IgE antibodies bind to specific receptors on cells such as basophils, mastocytes or Langerhans' cells. Upon allergen exposure, surface-bound IgE transduce a signal into the cell, which is followed by cell activation, which in the case of basophils and mastocytes is

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Such allergen-derived peptides containing one or a few T cell epitope(s) used in animal experiments and in human beings in an attempt to inhibit specific T cell activation and induce a state of T cell unresponsiveness, are described in the patent application WO93/08279.

One human application of this concept is the administration of a peptide derived from the sequence of T cell epitopes present on the Fel dI allergen, by subcutaneous injections in cat-sensitive individuals (Wallner B.P., Gefter M.L., *Allergy* 49, pp. 302-308 (1994)). An alternative, complementary approach of this concept has also been used in animal experiments. The peptides used are modified in such a manner as to keep the ability to bind to MHC-class II determinants on specific B cells, but which have lost their capacity to activate the corresponding T cells (O'Hehir R.E. et al., *International Immunology* 3, pp. 819-826 (1991)).

It is known that allergic reactions are generated by the liberation of mediators from target cells, such as basophils or mastocytes, having high-affinity surface receptors for IgE, which are occupied by IgE antibodies. The minimum requirement for mediator liberation to occur is that two IgE molecules recognising the same allergen are cross-linked, which in turn cross-link the receptor, resulting in the transduction of an activating signal within the cell. If only one IgE molecule is able to bind the allergen, no cell activation ensues, but the binding site of the IgE would be occupied, preventing cell activation upon exposure to native allergen. The use of a single IgE-binding epitope has therefore been claimed to be a suitable approach for the treatment of allergic diseases

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(Ball T. et al., *J. Biol. Chem.* 269, pp. 28323-28328 (1994), EP-A-0714662).

~~State of the art~~

~~The~~ US patent 4,946,945 describes a protein conjugate useful in immunotherapy, composed of a biological response modifier (BRM) and an allergen. Said conjugate could be combined with a pharmaceutically acceptable carrier. Cytokin, bacterial, fungal and viral immunopotentiators and thymus hormones are disclosed as suitable BRMs for use in said document.

The patent application WO95/31480 describes the preparation and the use of a synthetic compound made of two alpha-helices with specific arrangements of various amino acids. Said compound is used as a support for the binding of functional units, especially epitopes B and/or T.

~~Definitions~~

It is meant by "atopy", a predisposition, partly of genetic origin, of an individual having an immune system producing an excess of antibodies belonging to the IgE isotype in response to exposure to allergens. Individuals presenting such characteristics are therefore called "atopics".

An "allergen" is defined as a substance, usually a macromolecule of proteic composition, which elicits the production of IgE antibodies in predisposed, preferably genetically disposed, individuals (atopics).

Similar definitions are presented in the following references : *Clin. Exp. Allergy*, No. 26, pp. 494-516 (1996); *Mol. Biol. of Allergy and Immunology, ed. R.*

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The compound according to the invention is in a linear or a cyclic form, with or without additional moieties used, for instance to block peptide - peptide interactions.

Advantageously, the allergen is selected from the group consisting of *Der pI* and *Der pII* of house dust mite *Dermatophagoides pteronyssinus*, the major antigen of *Aspergillus fumigatus*, the staphylococcal B enterotoxin (SEB) and the bovine β-lactoglobulin or the allergen described in the documents *Clin. Exp. Allergy*, No. 26, pp. 494-516 (1996); *Mol. Biol. of Allergy and Immunology, ed. R. Bush, Immunology and Allergy Clinics of North American Series* (August 1996).

Advantageously, in the compound according to the invention, the antigenic determinant of an antigen which triggers T cell activation is a T cell epitope (preferably a helper T cell epitope) of tetanus toxoid, diphtheria, mycobacterium, influenza or measles viruses antigens (other examples of said T cell epitopes are described in the table II of the document WO95/26365).

Preferably, the compound according to the invention is selected from the group consisting of the peptides having the following ~~aminoacid~~ amino acid sequences:

SEQ ID NO. 1 :

QYIKANSKFIGITELGGHEIKKVLVPGCHGS

SEQ ID NO. 2 :

HEIKKVLVPGCHGS

SEQ ID NO. 3 :

DQYIKANSKFIGITELGGQYIKANSKFIGITELSSCHGSEPCIIHRGKPFGGCHGSEPCIIHRGKPFSSCHGSEPCIIHRGKPFGGCHGSEPCIIHRGKPFSSCHGSEPCIIHRGKPFGGCHGSEPCIIHRGKPFSR

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spontaneously or only minimally recognised by the immune system of atopic individuals.

These diseases include rhinitis and sinusitis of allergic origin, bronchial asthma, atopic dermatitis, some forms of acute and chronic urticaria, gastro-intestinal syndromes associated with the ingestion of food allergens such as β-lactoglobulin, the so-called oro-pharyngeal syndrome of the same origin, anaphylactic reactions associated with drug hypersensitivity.

The present invention will be described in the following examples, in reference to the enclosed figures. These examples are presented as non-limiting illustrations of the various embodiments of the present invention.

Brief D Drawings

~~Short description of the figures~~

Figure 1 represents Balb/c mice immunised by two SC injections of rDer pII (10 µg in Freund's adjuvant) administered at an interval of 2 weeks. The mice were bled and the reactivity of antibodies was evaluated using a set of overlapping peptides covering the Der pII sequence or the T cell adjuvant (FIS). Mice recognising peptide 11 (see point 2 in the Figure) were further immunised twice with 10 µg of peptide 21 and shown to recognise now peptide 21 with a 50 % reduction in the concentration of antibodies to peptide 11 (point 3 in the Figure). Further administration of rDer pII maintains the reactivity to peptide 21, while further reducing the concentration of antibodies to peptide 11 (point 4).

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density gradient. The tubes are centrifuged for 20 min at 1,000 g. Cells from the interface are collected by aspiration and resuspended in RPMI, washed twice with the same medium and finally resuspended in the same medium at 10^6 cells/ml. Fifty µl containing 10 µg/ml of either peptide 11-22 or 22-33 diluted in medium are added for an incubation of 6 days at 37°C. A positive control with PHA (10 µg/ml) is added. Proliferation of T cells is determined by assessing the extend of bromo-uridine (BrdU) incorporation in cell DNA, using an antibody specific for BrdU. results are shown in absorbency at 490 nm. No T cell proliferation above background value can be seen with peptide 11-22.

Detailed description of the invention

Preferred Embodiments

Atopics as well as non-atopic subjects produce antibodies towards environmental allergens. These antibodies belong to all isotypes described so far, including IgE (Saint-Remy J.M.R. et al., *J. Immunol.* 43, pp. 338-347 (1988)). It is usually observed that atopic individuals produce 10 to 100-fold more IgE antibodies than non-atopic individuals, which can at least partly explain why atopics suffer from symptoms when encountering allergens to which they are sensitised.

It has been unexpectedly discovered that the antigenic determinants of allergens such as *Der pI* and *Der pII* - two of the main allergens of the house dust mite *Dermatophagoides pteronyssinus* - which are recognised by

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Characteristics of the peptide

1. The B cell epitope is not recognised by IgE antibodies

The peptide is not recognised by IgE antibodies made by individuals sensitive to the native protein. This is established by an immunoassay carried out as follows. The peptide is insolubilised on polystyrene microtitration plates and a panel of serum samples of atopic individuals sensitive to *Der pII* is added; the binding of specific IgE antibodies is detected by addition of an isotype-specific reagent.

Thus, a peptide (SEQ ID NO. 2) of the sequence HEIKKVLVPGCHGS corresponding to amino acids ~~aminoacids~~ 11-24 of Der pII is obtained with solid-phase synthesis using methods well known to those skilled in the art with a biotin moiety added at its amino-terminal end. The peptide is insolubilised on neutravidin-coated plates and allowed to react with the serum of atopic individual. Results of such an experiment are shown in Figure 2. Thus, the serum of an atopic individual with IgE antibodies towards Der pII was added to a neutravidin-coated plate which had been pre-incubated with 12-mer peptides covering the sequences 7-39 of Der pII with 11 amino acid ~~aminoacid~~ overlap. No binding above the background value was observed for any of the 22 peptides, indicating the absence of IgE antibodies capable to bind to such sequences.

2. The B cell epitope is recognised by IgG antibodies of non-atopic individuals

This was established using a similar assay procedure as described above for IgE antibodies, except that a goat anti-human IgG antibodies was used for the detection of IgG antibodies and that a 1/100 dilution of

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serum was used. Representative results of such an experiment are given in Figure 3, from which it can be seen that significant binding occurred in between ~~aminoacid~~ amino acid 11 and 24, as well as in between ~~aminoacid~~ amino acid 22 and 34. The 7-39 region of Der pII therefore contains two binding sites for IgG of non-atopic individuals.

3. The B cell epitope is not recognised by IgG antibodies of atopic individuals

This was established using an assay procedure identical to the above-described assay for non-atopic subjects, except that the serum is now obtained from Der pII-hypersensitive patients. The results as shown in Figure 4 indicate that IgG of atopic individuals do not bind to the 11-24 Der pII region. A minority of patients have antibodies reacting with the 8-19 peptide.

4. The 11-24 Der pII region does not contain a T cell epitope

This was established by T cell proliferation assays using methods well known for those skilled in the art *(see for instance Current Protocols in Immunology, eds Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM and Strober W, Chapter 3, Greene Publishing Associates & John Wiley & Sons, 1992-1998)*. Peripheral blood mononucleated cells (PBMC) are separated from whole blood by density gradient centrifugation. The PBMC suspension is then incubated for 4 to 6 days with either rDer pII or with a 12-mer peptide included in the 7-39 region of Der pII. Results shown in Figure 5 indicate that addition of peptide 11-22 to the PBMC suspension did not result in proliferation of T cells, whereas significant proliferation

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was observed with peptide 22-33 and with PHA, the latter being used as a positive control.

Use of the hybrid peptide

The peptide (SEQ ID NO. 1) is mixed with an adjuvant suitable for human administration in order to increase its immunogenicity. Thus, muramyl-dipeptide (MDP) is used and covalently coupled to the peptide according to published methods (Matsumoto K. et al., *Immunostimulants: Now and Tomorrow*, Eds I. Azuma and G. Jolles, pp. 79-97 (1987), Japan Sci. Soc. Press, Tokyo/Springer-Verlag, Berlin).

The mixture containing the peptide and MDP is then administered to a patient sensitive to *Der pII*. Thus, a suspension containing 100 μg/ml of peptide in made in saline containing 0.3 % human serum albumin and 0.4 % phenol. One ml of the solution is injected in the arm by the subcutaneous route.

Example 2

The compound of the invention can be prepared by recombinant cDNA technology to produce a polypeptide made of a series of repetitive units of T and B cell epitope-containing peptides. A polypeptide made of a duplicated T cell epitope derived from TT (amino acids 830 to 844 of the heavy chain) and six repetitive B cell epitopes derived from Der pII is produced by DNA technology. A sequence of two amino acid residues is inserted in between each epitope. The sequence is:

D - $(QYIKANSKFIGITELX)_2$ - $(CHGSEPCIIHRGKPFX)_5$ - CHGSEPCIIHRGKPFSR, in which X is GG or SS.

(SEQ ID NO. 3)

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epitope of *Der pI* is obtained by synthesis. The sequence is PKYVKQNTLKLATGKKGPKYVKQNTLKLATGKKGVIIGIK (SEQ ID NO. 4).

The same characteristics as in example 1 are demonstrated using similar assay systems.

Example 5

The wild-type sequence of the B cell epitope-containing moiety can be altered in such a way as to eliminate an intrinsic T cell epitope while maintaining full immunogenicity of the B determinant, thanks to the presence of another functional T cell epitope within the immunizing peptide.

Thus, a 32 amino-acid long peptide of sequence QYIKANSKFIGITELGGCHGSEPCNIHRGKPF (sequence ID no. 5) is produced by synthesis as in Example 1. This peptide corresponds to a T cell epitope derived from TT (amino acid 830 to 844) and a B cell epitope derived from Der pII separated by a stretch of GG. The B cell epitope sequence has a point substitution in position 28, i.e. a substitution of I to N, which was shown to eliminate a major T cell epitope by assay systems as described in Figure 5.

The peptide is used for mouse immunization. Thus, six ~~Balb~~ BALB/c mice are injected in each footpad with 50 µl of an emulsion containing 50 µg of the peptide in complete Freund's adjuvant. The same injection procedure is used twice at a fortnight interval, except for the use of incomplete Freund's adjuvant. Two weeks after the last injection, the mice are bled and the serum shown to contain specific antibodies to the Der pII B cell epitope included in the synthetic peptide used for immunization, and to full-length Der pII protein. Regional draining lymph nodes

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are obtained for the preparation of T cell suspension. The latter are shown to proliferate in the presence of TT, but not in the presence of Der pII or the peptide corresponding to the B cell moiety used for immunization.

Example 6

Multiple antigenic peptides can be used for immunization with the advantage of increased immunogenicity and the possibility of using an immunogen containing B epitopes derived from different, possibly unrelated allergen molecules. Multiple antigenic peptides, or branched peptides, are synthesized according to methods known by those skilled in the art. Appropriate description of the methodology can be found for instance in Tam J.P., Proc.Natl.Acad.Sci USA 1988; 85: 5409-5413.

A core peptide made of 8 lysine (K) residues is made synthetically. Each K epsilon-amine group can be substituted by a particular peptide attached to the K backbone by a peptidic link. Thus, the first 2 residues are substituted with the sequence QYIKANSKFIGITEL (SEQ ID NO. 13) corresponding to the T cell epitope of TT (amino acid 830 to 844). Residues 3 and 4 are substituted with the sequence CHGSEPCNIHRGKPF (SEQ ID NO. 14) corresponding to the Der pII-derived B cell epitope with a I28N point substitution. Residues 5 and 6 are substituted with the sequence VIIGIK containing a B cell epitope derived from Der pI as shown in Example 4. Residues 7 and 8 are substituted with the sequence PKYVKQNTLKLAT (SEQ ID NO. 15) corresponding to a major T cell epitope of the influenza A virus.

The substituted branched peptide is used to immunize ~~Balb/c~~ BALB/C mice by the same procedure as described in

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Example 5. The serum is shown to contain antibodies to full-length Der pII and Der pI proteins and to the two B cell epitopes derived from these two allergens. T cell proliferation assays show a positive response to TT and to the influenza A viral protein containing the T cell epitope sequence.

Example 7

The nucleotide sequence coding for compound of the invention can be administered by gene transfer technology using recombinant viral or non-viral vectors (e.g. artificial lipid bilayers), molecular conjugates or modified recombinant organisms derived for instance from salmonella or mycobacteria.

Thus, an adenoviral vehicle containing the same DNA sequence as in Example 3 is engineered. This vector is prepared from two components: adenoviral DNA vector (Ad5 E1-E3-) and a packaging cell line. The sequences coding for one T cell epitope and two B cell epitopes are first inserted into the pAd plasmid. The linearized chimeric plasmid is then co-transfected using conventional DNA transfer techniques with the restricted Ad genoma into E1 transcomplementing 293 packaging cells for in vivo homologous recombination.

Viral stock prepared in 293 cells give titers ranged from $3x10^{10}$ to $2x10^{11}$ plaque-forming units per ml (pfu/ml).

10^7 pfus are administered by inhalation in ~~Balb/c~~ BALB/c mice. Mice are bled three weeks after and the level of antibodies towards ~~Derp~~ Der p II, and the B cell moiety

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contained in the immunizing construct is evaluated by direct binding ELISA as in Figure 3.

Example 8

The immunogenicity for humans of the compound of the invention can be evaluated in a humanized animal model. Thus, severe combined immunodeficiency (SCID) mice are reconstituted with immunocompetent cells of human origin. Peripheral blood mononuclear cells (PBMC; $15x10^6$ per mouse) obtained from an atopic donor sensitive to Der pII are injected into the peritoneum of each SCID mouse. Six mice reconstituted in such a way are injected at day 1, 15 and 30 with 50 μg of the recombinant polypeptide described in example 2. Mice are bled before and six weeks after the start of the immunization procedure. The serum is evaluated for the presence of antibodies to the recombinant polypeptide and found negative before and positive after immunization using a direct binding assay similar to that described in Figure 4.

Example 9: Cosmetic composition for skin hygiene

	% weight
Oil phase	
BRIJ 721 (Steareth 21)	4.00
Cetyl alcohol	10.00
Mineral oil	5.00
Propyl parahydroxybenzoate	0.02
Water phase	
CARBOPOL 943 (Carbomer 934)	0.10
Sodium hydroxide (solution at 10%)	0.10
Methyl parahydroxybenzoate	0.18
Compounds according to the example 1 to 3	0.50-5.00
Demineralised water	75.60-80.10
Total :	**100**

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ABSTRACT

COMPOUND AND METHOD FOR THE PREVENTION AND/OR THE TREATMENT OF ALLERGY

The present invention is related to a compound for the prevention and/or the treatment of allergy consisting of :

- at least one allergen antigenic determinant which is recognised by a B cell or an antibody secreted by a B cell of a non-atopic individual to said allergen, and
- at least one antigenic determinant of an antigen different from said allergen which triggers T cell activation.

~~(Figure 1)~~

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